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Novel pyranopyrazole derivatives comprising a benzoxazole core as antimicrobial inhibitors: Design, synthesis, microbial resistance and machine aided results

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ABSTRACT

From a medical point of view lot of existing antibiotics became unusable because microbial gained strong antibiotic resistance. The combination of two compounds in one core may lead to kill such type of pathogens. Herein, we developed pyranopyrazole derivatives comprising benzoxazole moiety by green approach strategy and studied their antimicrobial performance on four bacteria and two fungi. As a result, most of the compounds delivered reliable toxicity to kill the pathogens. In those, **6a** exhibited considerable activity against the microbial pathogens. Moreover, compounds **6d, 6l,** and **6n** showed prominent antibacterial activity. In addition, molecular docking studies of docked compounds revealed the strong bonding interaction with DNA-Gyrase and were docked into the intercalation location of DNA of the DNA-gyrase complex. The molecule bounded to the DNA stabilized by the H bonds, hydrophobic interactions, and π-π interaction. In addition, the linked 5-chlorobenazoxazole structure stabilized by the DT-8 and DG2009 of the F chain with pi-pi interactions. From the computer-aided results, it was observed that compound **6a** demonstrated maximum docking score −10.0 kcal/mole towards DNA-gyrase. Overall, this investigation suggested that these biologically active compounds can be utilized as leads for preclinical studies with the goal of developing newer antimicrobial drugs.

1. Introduction

Seventy years ago, before the introduction of antibiotics as drugs, microbial infections denoted a key reason of death. Unluckily, antibiotic resistance has grown-up and is nowadays spreading rapidly inspite of introduction of novel active antimicrobial medicines into the market, possibly leading to a health emergency in the nearby future [1]. Nowadays, a lot of infections happen to cause by microorganisms that are unaffected to most of the antibacterial drugs presently available [2]. It is expected that 70% of clinically isolated microbes are opposed to the frequently recommended antibiotics including methicillin along with additional b-lactam microbial resistant, like oxacillin and ampicillin [3,4]. Interestingly, vancomycin antibiotic drug was resisted by some microbial strains like *Enterococcus faecium* and *Staphylococcus aureus* [5]. The WHO (World Health Organization) recently circulated a list of antimicrobial-resistant 'priority pathogens' that involve a particular risk to community health. The vital goal of WHO is to guide and encourage the investigation and progress of innovative antimicrobials to benefit tackle worldwide antimicrobial resistance. [6]. In this context, the development of innovative biologically active combinations under an environmentally friendly path is one of the most important and successful strategy. Consequently, eco-catalyst, nontoxic reagents, experimental simplicity, cost minimization and less hazardous solvent medium are the greenest synthetic key points [7,8]. Scientific works have also contributed to the growing pollutions and therefore harming nature. Keeping the above understandings in mind, the current work bis heterocyclic composites and their synthesis has been taken up for the development of antimicrobials. A lot of compounds are known to contain azole structural moieties with antimicrobial activity such as Ketoconazole, Fluconazole, Voriconazole, Itraconazole, Posaconazole and more [9–12]. Due to the increasing resistance power of microbes, these drugs become inefficient. There are some more synthetic heterocyclic compounds which contain two nitrogen possess significant antimicrobial activities. Among them, pyranopyrazoles and benzoxazoles

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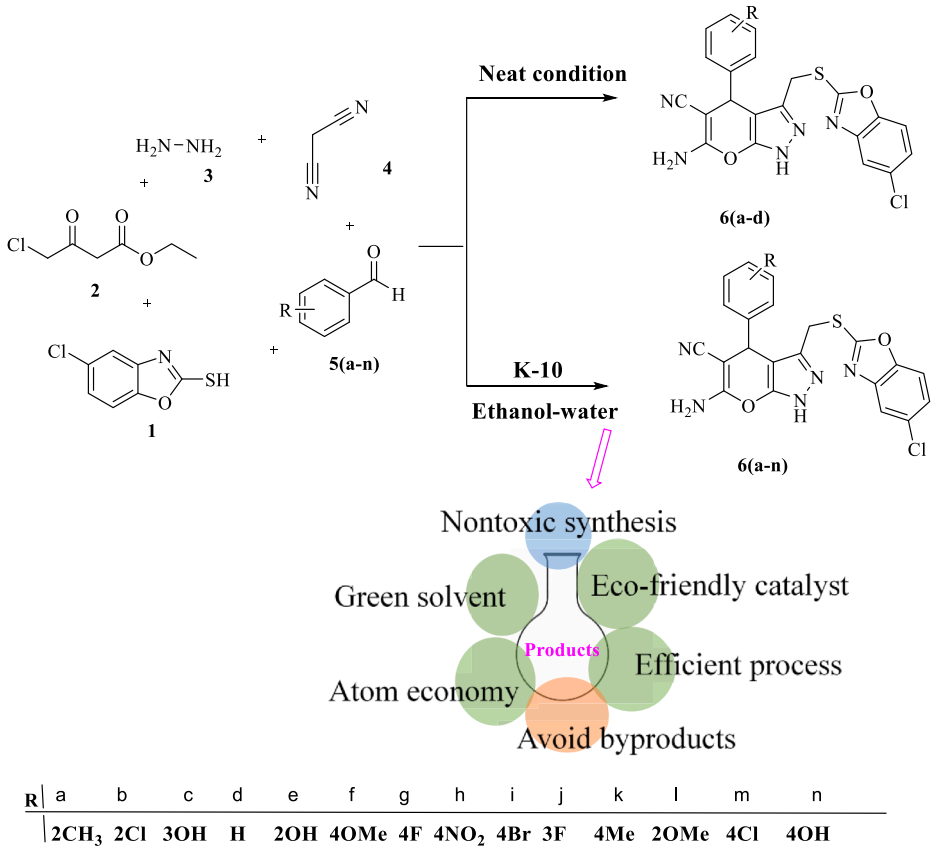


Scheme 1. Synthesis of pyranopyrazole linked benzoxazole derivatives.

Table 1
The *in-vitro* toxic values of prepared moieties **6(a–n)** on bacteria.

Samples	Zone of inhibition (mm)							
	Gram-positive				Gram-negative			
	S. aureus (ATCC-19433)		**B. subtilis** (ATCC-6633)		**P. vulgaris** (ATCC-29213)		**E. coli** (ATCC-8739)	
	25 µg/well	**50** µg/well	**25** µg/well	**50** µg/well	**25** µg/well	**50** µg/well	**25** µg/well	**50** µg/well
6a	32 ± 2	35 ± 1	29 ± 2	33 ± 1	25 ± 2	31 ± 1	22 ± 1	25 ± 1
6b	16 ± 1	18 ± 3	11 ± 1	12 ± 2	10 ± 2	13 ± 3	08 ± 2	11 ± 3
6c	22 ± 1	23 ± 1	15 ± 1	17 ± 3	15 ± 1	18 ± 1	10 ± 1	14 ± 1
6d	30 ± 3	33 ± 2	26 ± 3	28 ± 1	24 ± 3	29 ± 2	19 ± 1	23 ± 2
6e	19 ± 2	22 ± 1	13 ± 2	14 ± 2	13 ± 1	15 ± 1	09 ± 1	13 ± 1
6f	23 ± 3	25 ± 3	18 ± 2	20 ± 2	16 ± 2	21 ± 1	11 ± 2	16 ± 2
6g	03 ± 1	04 ± 3	02 ± 1	02 ± 3	02 ± 1	01 ± 1	01 ± 1	03 ± 2
6h	05 ± 1	08 ± 2	02 ± 1	04 ± 3	04 ± 2	05 ± 1	03 ± 2	04 ± 3
6i	15 ± 3	16 ± 2	08 ± 2	10 ± 1	09 ± 1	12 ± 2	07 ± 2	10 ± 2
6j	12 ± 2	14 ± 1	05 ± 3	07 ± 3	06 ± 2	10 ± 3	05 ± 1	09 ± 1
6k	25 ± 1	26 ± 1	19 ± 1	21 ± 3	19 ± 3	24 ± 2	12 ± 1	18 ± 1
6l	27 ± 2	29 ± 1	22 ± 1	26 ± 1	21 ± 1	26 ± 2	15 ± 1	19 ± 2
6m	09 ± 2	11 ± 2	03 ± 1	04 ± 1	05 ± 1	08 ± 1	05 ± 1	08 ± 2
6n	28 ± 1	30 ± 1	25 ± 1	27 ± 2	22 ± 1	28 ± 1	17 ± 3	21 ± 1
Ref	34 ± 2	37 ± 1	30 ± 1	35 ± 2	28 ± 1	32 ± 1	24 ± 2	27 ± 2
*	–	–	–	–	–	–	–	–

Ref = Ciprofloxacin; * = Control(DMSO).

are medicinally important moieties.

Benzoheterocycles like benzoxazoles are prominent heterocycles. Because in the medicinal chemistry point of view these compounds exhibited different types of medicinal properties [13–15]. Moreover, almost ten efficient drugs in the top two hundred medical products are benzoxazole related products [16]. Benzoxazole composites are key pharmacophore with no toxicities, which have delivered a diversity of medicinal activities [17–20]. Besides, the other compounds having two nitrogen elements are pyranopyrazoles. Pyranopyrazole and their derivatives revealed many biological properties such as anti-inflammatory [21,22], analgesic & antimicrobial [23,24] antibacterial [25] and anticancer [26]. A combination of the above two biological significance heterocycle structures in a single moiety delivers to the materialization of a fresh two heterocycle contained system which is prospective to exhibit novel biological activities by a synergistic influence. With our familiar knowledge in the field of bis heterocycles, not much work

Table 2
The *in-vitro* toxic values of prepared moieties **6(a–n)** on fungi.

Samples	Zone of inhibition (mm)			
	A. flavus (MTCC-1884)		A. niger (MTCC-1881)	
	25 μg/well	**50** μg/well	**25** μg/well	**50** μg/well
6a	35 ± 1	41 ± 3	28 ± 3	32 ± 2
6b	15 ± 2	19 ± 1	11 ± 1	14 ± 1
6c	19 ± 1	24 ± 3	15 ± 1	18 ± 1
6d	33 ± 3	38 ± 3	25 ± 2	30 ± 1
6e	18 ± 1	21 ± 2	13 ± 1	16 ± 2
6f	22 ± 2	26 ± 1	17 ± 3	20 ± 1
6g	04 ± 1	08 ± 3	02 ± 1	03 ± 1
6h	06 ± 3	11 ± 2	04 ± 1	05 ± 2
6i	14 ± 2	16 ± 2	10 ± 1	12 ± 2
6j	11 ± 1	15 ± 3	08 ± 1	09 ± 2
6k	24 ± 1	29 ± 1	18 ± 3	23 ± 3
6l	27 ± 1	32 ± 2	21 ± 2	25 ± 2
6m	09 ± 3	13 ± 3	06 ± 2	08 ± 3
6n	31 ± 3	35 ± 2	22 ± 3	26 ± 1
Ref	39 ± 1	43 ± 2	31 ± 1	36 ± 1
*	–	–	–	–

Ref = Ketoconazole, * = Control (DMSO).

reported in which benzoxazole and pyranopyrazole structures in one moiety. Thus, the current work describes the green synthesis of benzoxazole fused pyranopyrazoles and their biological evaluation. Furthermore, Molecular docking is also discussed.

2. Experimental

2.1. Chemistry

All the initial compounds, reagent and solvents were acquired and commercially available. Melting points verified by micro melting point device and were incorrect. Tetramethylsilane (TMS) as an internal standard (δ =0) for ^{1}H NMR, and for ^{13}C NMR, $CDCl_3$ ($\delta = 77.27$) was used as internal standard. For ^{1}H NMR: 500 MHz and for ^{13}C NMR: 75 MHz used. Carbon NMR spectra were obtained with complete proton decoupling. Low-resolution MS and HRMS data were obtained using ESI ionization.

2.1.1. Neat reaction procedure for synthetic routes of *6a, 6b, 6c* and *6d*

The four starting reagents, 5-chlorobenzo[*d*]oxazole-2-thiol (**1a,** one mmol), ethyl 4-chloro-3-oxobutanoate (**2,** one mmol), hydrazine (**3,** one mmol), malononitrile (**4**, one mmol) and 2-methylbenzaldehyde (**5a**, one mmol) were taken in a round bottom flask and proceeded the reaction at 60°under inert atmosphere for 5–7 hrs. After the completion of reaction checked by TLC, the product **6a** was isolated by using chromatography technique and ethyl acetate-hexane solvent mixture as eluent. The parallel procedure was followed to prepare **6(b–d)**.

2.1.2. Solvent and catalyst used preparation procedure of targets *6(a–n)*

To all starting compounds of product **6a,** solvent water–ethanol (2:1) in the volume of 5 mL was added followed by the addition of green montmorillonite *K*10 catalyst (half percentage by mass virtual to **5a**). The total set up kept at 60–70°temperature for 5–7 hrs. After TLC check, the reaction mixture filtered through funnel which contained cotton, the product contained filtrate solvent evaporated using rotary evaporate. The outcome product solid **6a** recrystallized with the help of methanol. The catalyst contained cotton dipped into ethyl acetate solvent. Consequently, catalyst settle down the bottom, decanted the solvent and the catalyst dried in oven at 50°. The recovered clay reused for further reactions. Similarly, the other targets **6(b–n)** were synthesized.

2.1.3. 4-(o-Tolyl)-3-(((5-chlorobenzo[d]oxazol-2-yl)thio)methyl)-6-amino-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (*6a*)

Light Yellow Powder; Yield 91%; m.p. 175–179 °C; IR (KBr) (cm^{-1}): 3437, 3342 (NH_2), 2242 (C≡N), 1668 (C═N) 1641 (C═C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.29 (s, 3H, CH_3), 3.61 (s, 2H, CH_2), 4.51 (s, 1H, CH), 7.34–7.70 (m, 7H, Ar-H), 8.56 (br, 2H, NH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.2 (Amine Attached C), 161.3 (N═C-S), 155.4 (O—C—NH), 150.9, 142.6, 135.2, 133.1, 132.2, 130.5, 129.6, 127.2, 126.9, 126.2, 124.5, 123.2, 122.5, 120.1 (Aromatic carbon), 109.3 (CN), 56.5(C-CN), 33.5 (CH), 29.2 (CH_2), 16.9 (CH_3). ppm; HRMS: *m/z* calcd for $C_{22}H_{17}ClN_5O_2S$ $(M + H)^+$ 450.0791; Found 450.0789.

2.1.4. 4-(2-Chlorophenyl)-3-(((5-chlorobenzo[d]oxazol-2-yl)thio)methyl)-6-amino-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (*6b*)

Light Yellow Powder; Yield 89%; m.p. 215–217 °C; IR (KBr) (cm^{-1}): 3442, 3339 (NH_2), 2241 (C≡N), 1660 (C═N) 1641 (C═C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.62 (s, 2H, CH_2), 4.58 (s, 1H, CH), 7.30–7.85 (m, 7H, Ar-H), 8.66 (br, 2H, NH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 169.0 (Amine Attached C), 161.5 (N═C-S), 156.3 (O—C—NH), 150.2 , 144.3, 135.4, 133.4, 131.6, 130.2, 128.4, 126.3, 125.9, 125.3, 124.5, 123.2, 122.1, 120.2 (Aromatic carbon), 110.3 (CN), 56.0 (C-CN), 36.1 (CH), 31.3 (CH_2). ppm; HRMS: *m/z* calcd for $C_{21}H_{14}Cl_2N_5O_2S$ $(M + H)^+$ 470.0245; Found 470.0241

2.1.5. 4-(3-hydroxyphenyl)-3-(((5-chlorobenzo[d]oxazol-2-yl)thio)methyl)-6-amino −1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (*6c*)

Yellow Powder; Yield 92%; m.p. 186–188 °C; IR (KBr) (cm^{-1}): 3445, 3331 (NH_2), 2230 (C≡N), 1674 (C═N) 1641 (C═C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.69 (s, 2H, CH_2), 4.59 (s, 1H, CH), 7.29–7.84 (m, 7H, Ar-H), 8.49 (br, 2H, NH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3 (Amine Attached C), 161.2 (N═C-S), 155.7 (O—C—NH), 151.6, 141.8, 135.4, 134.1, 133.2, 132.1, 130.9, 129.5, 128.3, 126.2, 125.2, 123.2, 121.5, 120.4 (Aromatic carbon), 109.5 (CN), 56.2 (C-CN), 35.5 (CH), 30.3 (CH_2). ppm; HRMS: *m/z* calcd for $C_{21}H_{15}ClN_5O_3S$ $(M + H)^+$ 452.0584; Found 452.0580.

2.1.6. 4-Phenyl-3-(((5-chlorobenzo[d]oxazol-2-yl)thio)methyl)-6-Amino-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (*6d*)

Light Yellow Powder; Yield 90%; m.p. 206–208 °C; IR (KBr) (cm^{-1}): 3442, 3330 (NH_2), 2235 (C≡N), 1669 (C═N) 1635 (C═C); ^{1}H NMR (500 MHz, $CDCl_3$): 3.69 (s, 2H, CH_2), 4.50 (s, 1H, CH), 7.19–7.84 (m, 8H, Ar-H), 8.54 (br, 2H, NH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3

Table 3
The MIC(MBC/MFC) of compounds **6d**, **6f** and **6g.**

Samples	MIC(MBC/MFC)					
	S. aureus	B. subtilis	P. vulgaris	E. coli	A. flavus	A. niger
6a	12.5 (25)	25 (50)	50 (200)	100 (>200)	25 (>200)	100 (>200)
6d	25 (50)	50 (200)	25 (100)	50(200)	25 (50)	50 (>200)
6n	25 (50)	25 (200)	100 (>200)	100 (>200)	50 (100)	25 (100)
Ref[1]	6.25	6.25	12.5	12.5	–	–
Ref[2]	–	–	–	–	6.25	12.5

Ref[1] = **Ciprofloxacin;** Ref[2] = **Ketoconazole.**

Table 4
Protein ligand interaction energies of ligands and active residues (chain in parenthesis) with DNA-gyrase calculated using binding analysis.

Molecule	Binding affinity (in kcal/mol)	Residues
6a	−10.0	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(E), Cyt-2012(E), Ade-2013(E), Ser-84(A), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B), Asn-476(B), Glu-477(B)
6d	−9.8	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(E), Cyt-2012(E), Ade-2013(E), Ser-84(A), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B), Asn-476(B), Glu-477(B)
6f	−8.6	Thy-8(F), Gua-2009(F), Cyt-2012(E), Ade-2013(E), Thy-2014(E), Arg-122(C), Arg-458(B), Gly-459 (B), Lys-460(B), Ile-461(B), Leu-462(B), Asn-475(B), Asn-476(B), Glu-477(B)
6g	−8.4	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2012(E), Ade-2013(E), Ser-84(A), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B), Asn-475(B), Asn-476(B), Glu-477(B)
6h	−8.5	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(F), Cyt-2012(E), Ade-2013(E), Ser-84(A), Arg-122(C), Asp-437(B), Arg-458(B), Asn-476(B)
6k	−8.9	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(E), Cyt-2012(E), Ade-2013(E), Ser-84(A), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B)
6l	−9.1	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(F), Cyt-2012(E), Ade-2013(E), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B), Asn-476(B), Glu-477(B)
6n	−9.8	Thy-8(F), Gua-2009(F), Gua-2010 (F), Cyt-2011(F), Cyt-2012(E), Ade-2013(E), Arg-122(C), Asp-437(B), Arg-458(B), Gly-459 (B), Asn-476(B), Glu-477(B)

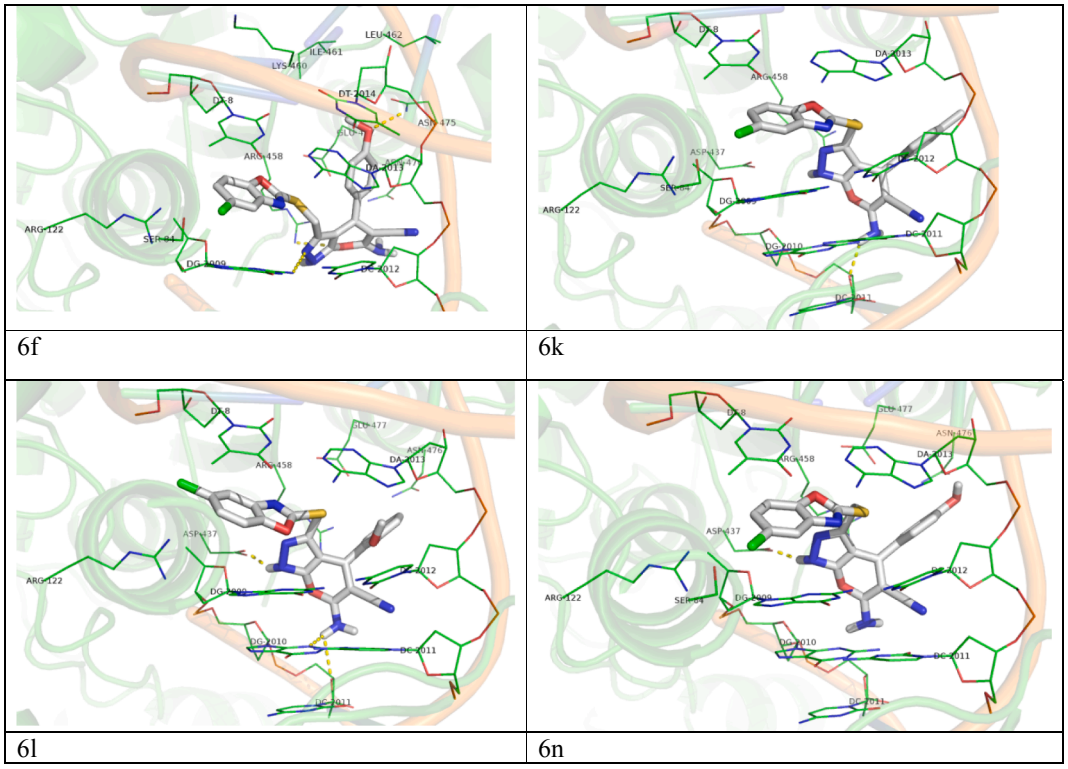


Fig. 1. Molecules **6f, 6k, 6l** and **6n** were docked in the active site of the DNA-gyrase cleavage complex of S. aureus (PDB_ID:5CDQ). Inhibitor molecules shown in white color stick style and the DNA side chains and amino acid side chains are shown in line style.

(Amine Attached C), 162.5 (N═C-S), 155.3 (O—C—NH), 150.3, 144.1, 138.2, 136.1, 132.2, 130.9, 126.4, 123.2, 122.5, 121.3, 120.5, 118.4 (Aromatic carbon), 110.2 (CN), 57.3 (C-CN), 35.4 (CH), 28.2 (CH_2). ppm; HRMS: *m/z* calcd for $C_{21}H_{15}ClN_5O_2S$ $(M + H)^+$ 436.0635; Found 436.0632.

2.2. Antimicorbial assay

By using four bacterias and two fungi pathogens, microbial toxicity results were screened according to the method sited there in [27]. For this, Ciprofloxacin and Ketoconazole were used as references and in 25 and 50 μg/well concentrations were used.

2.3. Molecular docking study

Molecular docking studies of molecules into the crystal structures of DNA-gyrase cleavage complex of S. aureus (Gram-positive bacteria) with PDB_ID:5CDQ were carried out using Autodock Vina software [28,29], open source molecular docking software. We have generated a grid box with desired parameters around the active site of DNA-gyrase cleavage complex of S. Aureus (PDB_ID:5CDQ) [30] as centre :x = 40.124, y = -46.732, z = 64.933 and grid box size: x = 22, y = 36, z = 26. We generated 20 conformations in each docking output by using advanced Genetic algorithm method in Vina. Protein/DNA complex and molecule input preparations and docking output analysis were carried out using MGLTools-1.5.6 software.

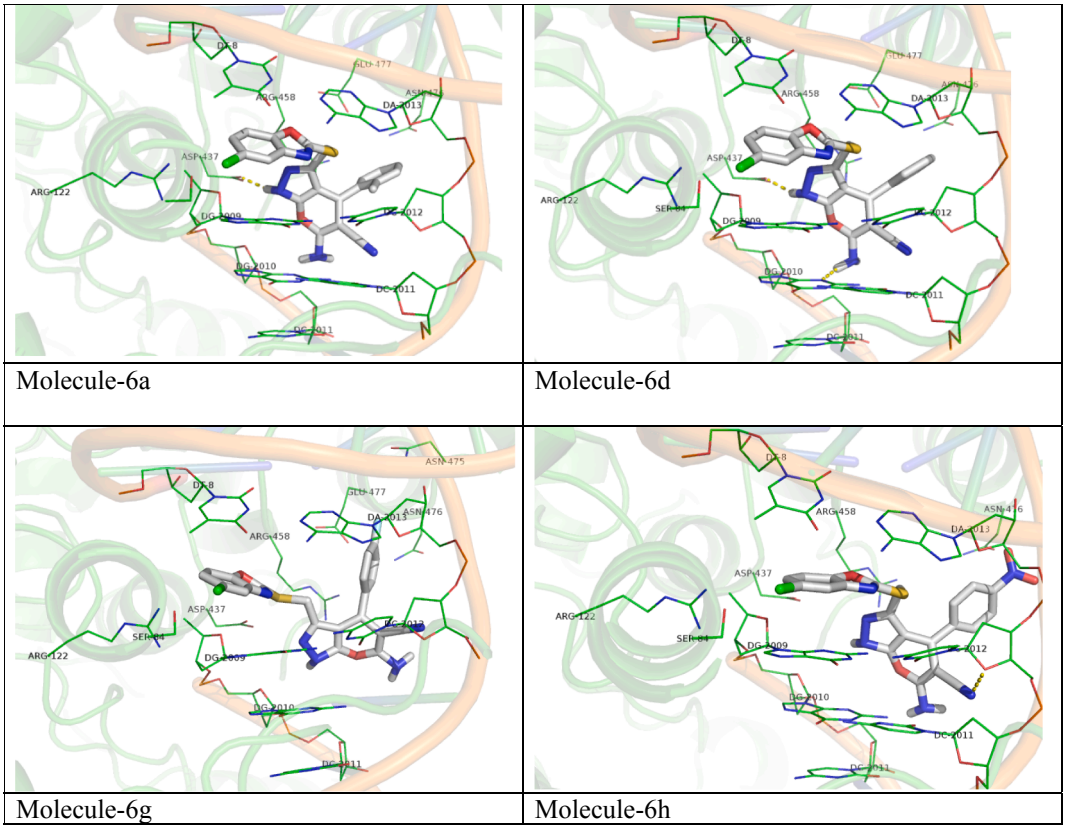


Fig. 2. Molecules **6a, 6d, 6g** and **6h** were docked in the active site of the DNA-gyrase cleavage complex of S. aureus (PDB_ID:5CDQ). Inhibitor molecules shown in white color stick style and the DNA side chains and amino acid side chains are shown in line style.

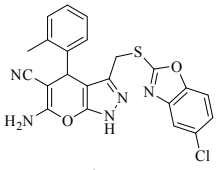
3. Results and discussion

3.1. Chemistry

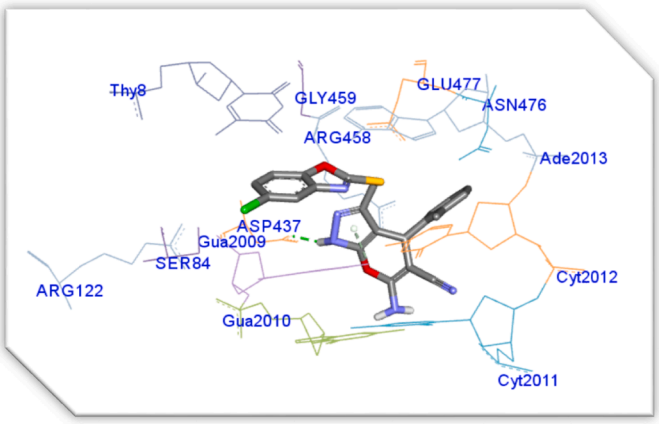
The simple and green synthetic method for the preparation of pyranopyrazole attached benzoxazole and their derivatives were depicted in scheme 1. In addition mechanism of product formation was depicted in scheme 2. Foremost, few compounds **6a, 6b, 6c**, and **6d** were prepared in different eco-friendly methods to rationalize the best method. After, other targets **6(e-n)** were prepared *via* an effective technique. In this fashion, all starting compounds of product **6a** were mixed and kept the reaction without solvent and catalytic agent. As a result, **6a** was formed, but only 32% yield was obtained along with byproduct (**7a**) (Scheme 3). This byproduct was formed due to the side reaction between aldehyde and hydrazine. The same situation existed in the case of targets **6b**, **6c** and **6d**, their outcome yields in this method were 28%, 34%, and 30%, correspondingly. In addition, side products (**7(b-d)**) also formed. The side product formation in this method may lead to low yield of the product. The other cause of the trace profits may be due to deficiency of solvent or reagent or both. Further, to obtain higher yields of the product along with sustaining the ecologically benign condition, we decided to use the montmorillonite *K*10 catalyst and solvent. When K 10 catalyst and a green solvent such as a mixture of water–ethanol (2:1) were added to the flask which already contained total preliminary compounds of product **6a** and proceeded reaction again. Subsequently, **6a** was formed with a 91% yield. In the identical technique, the amalgams **6b**, **6c**, and **6d** resulted 89, 92 and 90% of yields. That means outstanding results were observed in this method. Moreover, absent of byproduct formation was observed in this second method. The reason may be due to the presence of a solvent that will help to mix the reactants clearly and the spur which promotes all initial compounds to target, as a result, yields come quantitatively. Based on the optimized conditions, the rest of the products **6(e–n)** were prepared by this method, resulted in a high yield of the products. Interestingly, the spur *K*10 catalyst was recycled up to five times and used for other reactions. This was the additional advantage of this eco-friendly method over other methods having similar type of molecules [31,32]. Moreover, synthetic method under environmental favor conditions is the most valuable research method over other. In addition, no harmful catalysts were used in this method. Compare to other reported methods, using of eco-friendly solvent media and quantitative yield of the product are the most advantageous tools that are existed in this method

3.2. Compounds toxic effect on microbial

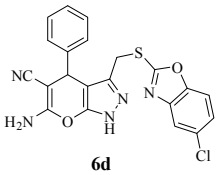
To detect any toxicity behavior of above synthetic molecules on microbes the available four bacteria and two fungi (Table 1) were chosen. The antimicrobial screening of all synthesized conjugates against four bacteria are tabulated in Table 1. We were delighted to perceive that from the bacterial point of view all compounds **6(a–n)** delivered reliable toxicity nature. In those, some compounds exhibited excellent antibacterial nature on four bacteria, few compounds displayed moderate antibacterial performance and few compounds behaved mild antibacterial potential towards four bacteria. Captivatingly, all **6(a–n)** products exhibited excellent antibacterial nature against Gram-positive bacteria only. Also, against *S. aureus*, these compounds displayed good antibacterial performance against *S. aureus* bacteria. When we observed the competition between the active compounds regarding the toxicity, *ortho* methyl substitution on benzene ring moiety gave the highest killing nature towards all bacteria. Its antibacterial results were closed to reference drug Ciprofloxacin. Furthermore, **6d, 6 l**, and **6n** screened compounds showed prominent antibacterial activity, while the other moieties displayed moderate to least activities.



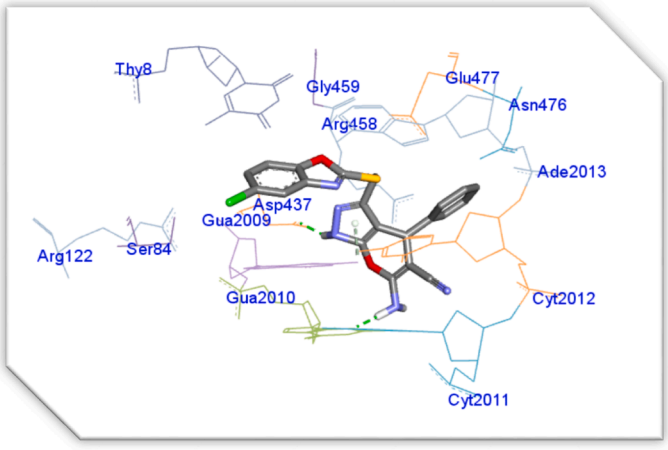
6a
Docking score -10.0 kcal/mol



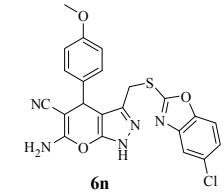
6a



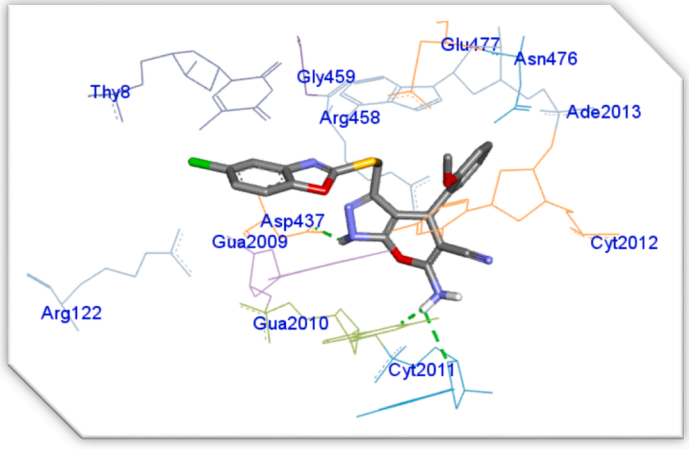
6d
Docking score -9.8 kcal/mol



6d



6n
Docking score -9.8 kcal/mol



6n

Fig. 3. Binding affinity and their energies of compounds **6a, 6d** and **6n.**

These compounds were also evaluated against two fungi such as *A. nigar* and *A. flavus* and the results are represented in Table 2. The deadliness examination was only authenticated up to two meditations of 25 and 50 μg per well and suits our purpose to detect prominent antifungal active compounds. Molecules tangled within this investigation (**6(a–n)**) were determined to be high to minimal toxic contrary to the two fungal strains. Moreover, total compounds were displayed high toxic nature towards A. niger than other fungi. Besides, molecule **6a** holds most deadliness than other molecules, while the composites **6d, 6f, 6k**, **6l,** and **6n** displayed good to moderate

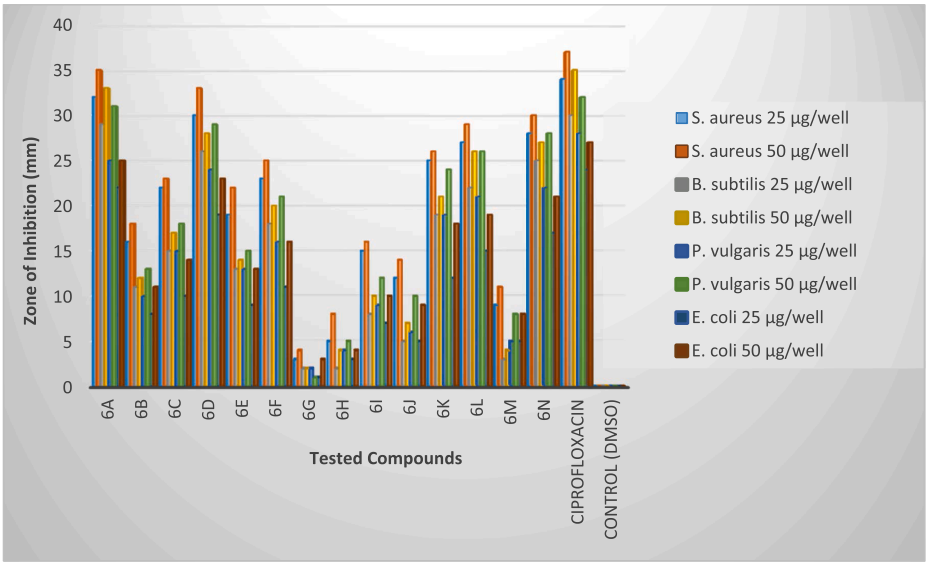


Fig. 4. Graphical representation of *in vitro* toxicity nature of all compounds against bacteria.

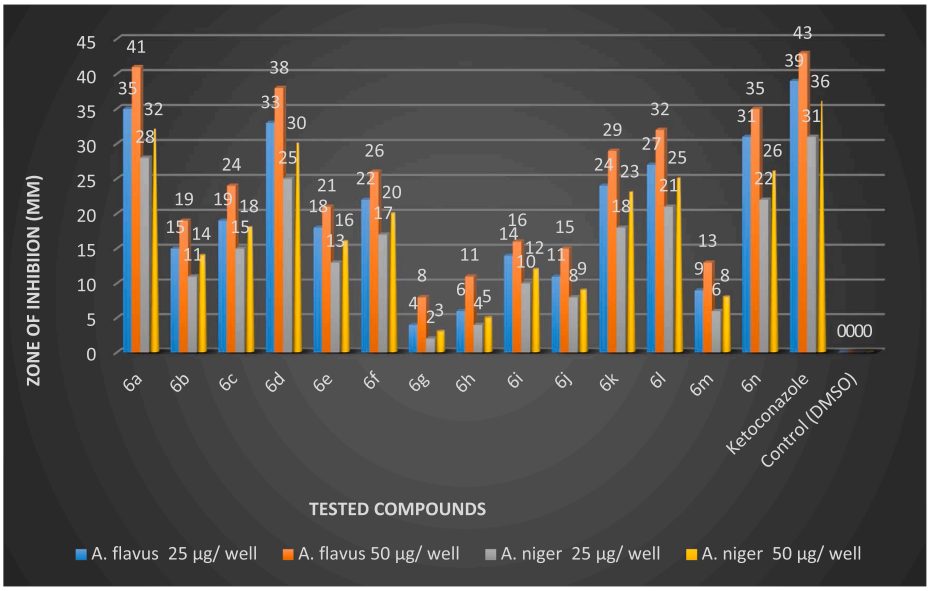


Fig. 5. Graphical representation of *in vitro* toxicity nature of all compounds against fungi.

antifungal properties. On the other hand, remaining molecules contained low to least poisonousness towards fungi

3.3. Minimum inhibitory concentration (MIC) and minimum bactericidal/ fungicidal concentration (MBC/MFC)

To estimate the MIC and MBC/MFC, most effective compounds **6a, 6d** and**6n** were preferred because of their strong antibacterial effect on all bacterial and fungal pathogens which were mentioned above. From the tabulated grades (Table 3), **6a** exhibited lowest bacterial MBC result equal to 2X lowest inhibitory volume (i.e.MIC) towards both Gram-positive germs, whereas, **6d** and **6n** have 2X lowest inhibitory concentration (i.e.MIC), which was equal to lowest bacterial volume (i.e.MBC) value against S. aureus pathogen only. It was detected that three composites delivered the smallest volumes to kill the Gram-positive pathogens only. Also, the smallest fungicidal volume (i.e. MFC) which was equal to double to lowest inhibitory concentration (i.e. 2X MIC) happened. This result belongs to **6d** and **6n** against A. flavus pathogen. Meanwhile, the other target **6a** displayed the lowest bacterial/ fungicidal volume of more than 2X lowest inhibitory concentration (i.e.MIC).

3.4. Molecular docking studies and SARs analysis

The binding affinity of eight molecules are shown in table 4, towards the DNA-gyrase cleavage complex of S. aureus DNA binding site surrounded by the protein residues, was analyzed using molecular docking studies. The binding affinity of the molecule showing strong interaction energies with the DNA active site and the results were shown in table 4.

The crystal structure of the DNA-gyrase cleavage complex of *S. aureus* (PDB_ID:5CDQ) has been considering for the docking of the eight molecule**s** in Table 4. The crystal structure contains an inhibitor Moxifloxacin intercalation in the E and F chains of DNA. The DNA binding protein with A and C chains are stabilizing the binding of the DNA with the bound Moxifloxacin. Compounds were docked into the intercalation site of DNA of the DNA-gyrase complex. The binding to the DNA was stabilized by the hydrogen bonds, hydrophobic interactions, and Pi-Pi

interaction. In all the eight molecules, the side chain 5-chlorobenazoxazole ring stabilized by the thymine ring (DT-8) and Guanine (DG2009) of the F chain with pi-pi interactions. The 1,4 dihydropyranopyrazole ring NH is forming a stable hydrogen bond with side-chain carboxylic oxygen of ASP-437 of B chain, it was observed in active molecules **6a**, **6d**, **6l**, and **6n** (Fig. 1&2). The phenyl ring of molecule **6d**, 2-methyl phenyl of **6a** and 4-hydroxy phenyl of **6n** substituted on dihydropyranopyrazole ring stabilized by the B chain amino acid residues ASN-476, ARG-458, GLU-477. The molecule **6n** cyanide nitrogen forming hydrogen bond with the oxygen atom of E chain cytosine sugar ring. The electron-withdrawing groups $–NO_2$, -OMe, -F on the phenyl ring is stabilized by only ASN476. The loss of hydrogen bond with ASP-437 observed in molecules **6g**, **6h**, **6k**, and **6f** may cause a decrease in the activity of these molecules. The interaction of DNA bases and the amino acid side-chains proteins with active **6a** and **6d** molecules and less active molecules **6g** and **6h** were shown in Fig. 2

With biological evaluation and docking results in hand, it is necessary to determine the structural behavior of active compounds like how structural orientation, any biological influence part in the structure, what parameters may force to get biological activeness of the molecule and other. Among all results, compound **6a** showed maximum docking score (− 10.0 kcal/mol) and it delivered high toxicity nature to kill the microbial (Fig. 3). That means the maximum binding energy nature of this compound may lead to stand highest antimicrobial property. The next leading binding energy compounds were **6d** and **6n.** In this case, even though both have equal docking score values of − 9.8 kcal/mol, compound **6d** delivered second-highest toxicity values on microbial than **6n** (Fig. 4 & Fig. 5**)**. This may be due to the presence of the hydroxyl group in **6n** that controls the toxic nature of the compound.

3.5. Conclusion

New series Pyranopyrazole linked benzoxazole derivatives were reported in this research. The eco-friendly synthetic tactic was used to obtain the target compounds. Consequently, quantitative yield of the products obtained. Further, total targets were analyzed about their antimicrobial potential on bacterial and fungi strains. All the compounds showed high to least via moderate antimicrobial properties. In addition, all compounds were active against Gram-positive bacteria than others. Compound **6a** displayed maximum antimicrobial activity among all. In addition, compounds **6d, 6n** and **6 l** delivered good toxic property on microbial**.** The molecular docking studies of docked compounds delivered the strong bonding interaction with DNA-Gyrase and were docked into the intercalation location of DNA of the DNA-gyrase complex. The molecule bound to the DNA stabilized by the H bonds, hydrophobic interactions, and π-π interaction. In addition, the linked 5-chlorobenazoxazole structure stabilized by the DT-8 and DG2009 of the F chain with pi-pi interactions. Besides, docking results revealed that **6a** has the highest binding energy value of − 10.0 kcal/mole against the target enzyme.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103908.

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